EXHIBIT F

WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

N-NITROSO COMPOUNDS: OCCURRENCE AND BIOLOGICAL EFFECTS

Proceedings of the VIIth International Symposium on N-Nitroso Compounds held in Tokyo, 28 September – 1 October 1981

EDITORS

H. BARTSCH
I. K. O'NEILL
M. CASTEGNARO
M. OKADA

TECHNICAL EDITOR FOR IARC
W. DAVIS

IARC Scientific Publications No. 41

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER
LYON
1982

Seplae
PUBL

The International Agency for Research on Cancer (IARC) was established in 1965 by the World Health Assembly as an independently financed organization within the framework of the World Health Organization. The headquarters of the Agency are at Lyon, France.

The Agency conducts a programme of research concentrating particularly on the epidemiology of cancer and the study of potential carcinogens in the human environment. Its field studies are supplemented by biological and chemical research carried out in the Agency's laboratories in Lyon and, through collaborative research agreements, in national research institutions in many countries. The agency also conducts a programme for the education and training of personnel for cancer research.

The publications of the Agency are intended to contribute to the dissemination of authorative information on different aspects of cancer research.

I OUP ISBN 0-19-723041-5

150 cours Albert Thomas, 69372 Lyon Cedex 08, France

The authors alone are responsible for the views expressed in the signed articles in this publication.

PRINTED IN SWITZERLAND

R Case 7 ... 1981 Publ

CONTENTS

Foreword xiii

Introduction xv

List of Participants xvii

Members of the Conference Organizing Committee xliii

Overview xlv

CHEMISTRY AND FORMATION

A novel oxidative degradation of N-nitrosamine *via* electron transfer
Masui, M., Nose, K., Tanaka, A., Yamakawa, E. & Ohmori, H. 3

Amine nitration and nitrosation by gaseous nitrogen dioxide
Challis, B.C., Fine, D.H., Goff, E.U., Hoffman, G.A. & Shuker, D.E.G. 11

N-Nitrosation of secondary amines by nitric oxide *via* the 'Drago Complex'
Hansen, T.J., Croisy, A.F. & Keefer, L.K. 21

Formation of N-nitrosamines and N-nitramines by photolysis
Challis, B.C. & Li, B.F.-L. 31

Nitrosamines formation from ternary nitrogen compounds
Loeppky, R.N., Tomasik, W., Outram, J.R. & Feicht, A. 41

Nitrosation of N-methyltyramine and N-methyl-3-aminomethylindole, two barley malt alkaloids
Mangino, M.M., Libbey, L.M. & Scanlan, R.A. 57

Preussmann, R., Spiegelhalder, B., Eisenbrand, G., Würtele, G. & Hofmann, I. (1981) Urinary excretion of *N*-nitrosodiethanolamine in rats following its epicutaneous and intratracheal administration and its *in vivo* formation following skin application of diethanolamine. *Cancer Lett. 13*, 227-231

Schmeltz, I., Abidi, S. & Hoffmann, D. (1977) Tumorigenic agents in unburnt proceeded tobacco: *N*-nitrosodiethanolamine and 1,1-dimethylhydrazine. *Cancer Lett., 2*, 125-132

Zweig, G., Selim, S., Hummel, R., Mittelman, A., Wright, D.P., Law, C. & Regelman, E. (1980) *Analytical survey of N-nitroso contaminants in pesticide products.* In: Walker, E.A., Griciute, L., Castegnaro, M. & Börzsönyi, M., eds. *N-nitroso Compounds: Analysis, Formation and Occurrence.* Lyon, International Agency for Research on Cancer (*IARC Scientific Publication No. 31*), pp. 555-562

INDUCTION OF PRENEOPLASTIC AND NEOPLASTIC LESIONS IN RATS TREATED *N*-NITROSO COMPOUNDS

N. ITO, S. FUKUSHIMA, H. TSUDA & T. SHIRAI

First Department of Pathology, Nagoya City University Medical School, Mizuho-ku, Nagoya 467, Japan

M. ARAI

Department of Pathology, School of Hygiene, Fujita-Gakuen University, Dengakugakubo, Aichi 470-11, Japan

INTRODUCTION

It is well known that *N*-nitrosodimethylamine (NDMA) is a potent carcinogen for the liver and kidneys and that *N*-nitrosobutyl-4-hydroxybutylamine (NBHA) is carcinogenic for the urinary bladder. The present experiments were conducted to examine whether the incidences of preneoplastic and neoplastic lesions in the liver or urinary bladder in rats are dependent on the dose of NDMA or NBHA, respectively, and whether the results of short-term and long-term experiments show similar dose-response effects.

MATERIALS AND METHODS

Experimental Series I. Dose-dependent effect of NDMA on liver carcinogenesis.

In the short-term experiment, 7-week-old, male, Fischer 344 rats (Charles River Japan Inc., Kanagawa, Japan) were initiated by a single intraperitoneal injection of *N*-nitrosodiethylamine (NDEA) (Tokyo Chemical Industry Co., Tokyo) at a dose of 200 mg/kg body weight. Two weeks

after the initiation, the animals were placed on a powdered diet (Oriental M, Oriental Yeast Co., Ltd., Tokyo, Japan) containing NDMA (Tokyo Kasei Co.) at a concentration of 10.0, 1.0 or 0.1 mg/kg for 6 weeks. Some were on the basal diet as a control. Rats injected with only saline were given only the highest dose of NDMA, in the same manner. All animals were subjected to a partial hepatectomy on the first day of the fourth experimental week. All rats were sacrificed at the end of week 8 and the liver tissues were processed for γ-glutamyltranspeptidase (γ-GT) staining. Numbers and areas of γ-GT positive foci in the liver sections were determined with a color image processor (Olympus VIP 21C) and were expressed as the number and area (mm^2) per unit area of the liver section (cm^2).

In the long-term experiment, both sexes of Wistar strain rats (CLEA Japan Inc.), 6 weeks of age, were fed a NDMA-containing diet at levels of 10.0, 1.0 and 0.1 mg/kg for 96 weeks. Control rats were kept on the basal diet. All animals were killed in week 96 and liver tumours were examined histologically.

Experimental Series II. Dose-dependent effects of BBN on urinary bladder carcinogenesis.

In the short-term experiment, 6-week-old, male, Fischer 344 rats were continuously given NBHA (Izumi Chemical Co, Yokohama, Japan) in the drinking water at a concentration of 0.05, 0.025, 0.01, 0.005, 0.001 or 0.0005%. Five animals were sacrificed at each of weeks 0, 4, 8 and 12. The urinary bladders were fixed by an intraluminal injection of 2.5% glutaraldehyde solution. They were processed for histological examinations, as well as for SEM and transmission electron microscopical studies.

In addition, 8-week-old, male, Wistar strain rats (Shizuoka Animal Farm, Shizuoka, Japan) were continuously treated with 0.005, 0.001 or 0.0005% NBHA in the drinking water for up to 40 weeks. Animals were sacrificed at the end of weeks 20 and 40. The bladders were fixed with 10% buffered formalin and hematoxylin-eosin slides were prepared.

RESULTS AND DISCUSSION

Experimental Series I.

Short-term treatment with NDMA, coupled with partial hepatectomy after initiation with a single exposure to NDEA, induced a dose-related increase of the number and area of γ-GT positive foci in the liver (Table 1); significantly higher increases were seen at doses of 10.0 and 1.0 mg/kg[1], compared to the control values. A similar dose-related effect was also found in the induction of pre-neoplastic lesions in the

[1] All doses are expressed as mg/kg diet.

long-term treatment with NDMA alone, especially in male rats (Table 2). The long-term oral administration of NDMA developed not only hyperplastic nodule and hepatocellular carcinoma, but also hemangioendothelioma and fibrosarcoma at doses of 1.0 and 10.0 mg/kg. No renal tumours were found in this work. This tumorigenic activity of NDMA at doses of 1.0 and 10.0 mg/kg coincided with the results of the short-term experiment. Without initiation with NDEA, feeding of NDMA even at a dose of 10.0 mg/kg did not induce a significant increase in γ-GT positive foci. These findings suggest that the initiating activity of NDMA is relatively weak. This could be one of reasons why the administration of NDMA for up to 96 weeks at a dose of 0.1 mg/kg did not induce any neoplastic changes in the liver. The present results also suggest that the minimum carcinogenic dose of NDMA in food is 1.0 mg/kg and that the non-effective level is about 0.1 mg/kg, when orally administered to rats for 96 weeks.

Table 1. Dose-related effect for induction of γ-GT positive foci induced by short-term treatment with dietary NDMA in male F344 rats pretreated i.p. with NDEA

Group	Pretreatment with NDEA	Dose of NDMA (mg/kg)	No. of animals	γ-GT positive foci in the liver[a]	
				No./cm^2	Area (mm^2/cm^2)
1	+	10.0	20	10.3 ± 2.9[b]	0.30 ± 0.09[b]
2	+	1.0	20	6.1 ± 1.6[c]	0.23 ± 0.08[c]
3	+	0.1	19	4.1 ± 1.1	0.14 ± 0.05
4	-	10.0	17	< 0.2	< 0.01
5	+	0	21	4.0 ± 1.1	0.14 ± 0.06

[a] Mean ± S.D.

[b] Significantly different from groups 2, 3, 4 and 5.

[c] Significantly different from groups 3, 4 and 5.

Table 2. Development of tumours in male Wistar rats treated with NDMA for 96 weeks

Group	Dose of NDMA (mg/kg)	No. of rats	No. of rats with changes in liver[a]			
			Hyperplastic nodule	Hepato-cellular carcinoma	Haemangio-endo-thelioma	Fibro-sarcoma
1	10.0	17	6 (35.3)	1 (5.9)	3 (17.6)	5 (29.4)
2	1.0	15	1 (6.7)	1 (6.7)	0 -	1 (6.7)
3	0.1	9	0 -	0 -	0 -	0 -
4	0	7	0 -	0 -	0 -	0 -

[a] The percentage is given in parentheses.

Experimental Series II.

Incidences of three kinds of early pre-neoplastic lesions, such as pleomorphic microvilli, short and uniform microvilli and ropy or leafy microridges on the cell surface of the urinary bladder, observed by SEM showed a tendancy to increase with longer treatment and higher dose (0.1 to 0.5%) of NBHA. These changes observed by SEM correlated well with the incidences of pre-neoplastic lesions, such as simple hyperplasia and papillary or nodular hyperplasia. No lesions were detected at concentrations less than 0.005%, either by light microscope or by SEM. A clear dose-dependent effect was found for the incidence of papilloma and carcinoma in the groups subjected to the longer treatments at doses of 0.001% and 0.005%. There were no pathological changes in the urinary bladder in the group with 0.0005% NBHA. When the results of short-term and long-term experiments were compared, carcinomas were seen in the latter experiment at a dose of 0.005%, at which dose no observable changes except occasional ropy or leafy microridges were detected by SEM at week 12. This discrepancy may be due to the difference in rat strain and it may be suggested that the period of 12 weeks was too early to detect changes in the urothelium induced by a dose as low as 0.005% of NBHA. A similar dose-dependent increase of pre-neoplastic and neoplastic lesions of rat urinary bladder was reported in a previous work (Ito *et al.*, 1975).

The present results also confirm that the carcinogenicity of BBN for the urinary bladder depends on the dose and duration of treatment.

SUMMARY

Dose-related changes of incidences of pre-neoplastic and neoplastic lesions in the liver or urinary bladder were examined with *N*-nitroso-dimethylamine (NDMA) or *N*-nitrosobutyl-4-hydroxybutylamine (NBHA) respectively. The incidences of lesions by the two carcinogens were compared in short-term and long-term experiments.

In a short-term experiment, rats were given a basal diet containing NDMA at concentrations of 10.0, 1.0 and 0.1 mg/kg for 6 weeks, after initiation by a single i.p. injection of *N*-nitrosodiethylamine (NDEA). Similar, long-term experiments (96 weeks) were also performed, but without NDEA pretreatment. A clear dose-related effect was found in the induction of pre-neoplastic lesions when the groups given 10.0 and 1.0 mg/kg NDMA were compared. This coincided with the results of a long-term experiment on the induction of hyperplastic nodules and hepato-cellular carcinomas.

Various concentrations of 0.05 to 0.005% of NBHA in the drinking water were given for different periods up to 12 weeks, in a short-term, and up to 40 weeks in a long-term experiment. Incidences of early pre-neoplastic lesions of the cell surface observed by scanning electron microscopy showed a tendency to increase with longer duration and higher dose (0.01 to 0.05%). A clear dose-dependent effect was found for the incidence of papilloma and carcinoma in the groups subjected to longer treatments.

REFERENCE

Ito, N., Arai, M., Sugihara, S., Hirao, K., Makiura, S., Matayoshi, K. & Denda, A. (1975). Experimental urinary bladder tumours induced by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine. *GANN monograph on Cancer Research, 17*, 367-381